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(54) Title: AMYLIN ANTAGONISTS AND AGONISTS

(57) Abstract

The invention features amylin analogs which behave as amylin antagonists and agonists. The invention also features the use of the amylin antagonist for the treatment of Type II diabetes mellitus, and the use of the amylin agonists for the treatment of both Type I diabetes mellitus and hypercalcemia. The invention also features the use of amylin antagonists and agonists for the control of food intake.

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AMYLIN ANTAGONISTS AND AGONISTS

Background of the Invention

This invention relates to specific amylin analogs
5 which behave as amylin antagonists and agonists, and to
their use in the treatment of diabetes mellitus, and
hypercalcemia, and the control of food intake.

Amylin, also known as diabetes associated
polypeptide (Cooper et al., Proc. Natl. Acad. Sci. USA,
10 85:7763-7766 (1988)) or islet/insulinoma amyloid
polypeptide (Westermark et al., Proc. Natl. Acad. Sci.
USA, 84:3881-3885 (1987)), is a 37-residue polypeptide
amide isolated originally from the amyloid-rich pancreas
of insulinoma and noninsulin-dependent diabetic (NIDD)
15 patients. It has subsequently been isolated from the
normal pancreas of rat (Asai et al., Biochem. Biophys.
Res. Commun., 164:400-405 (1989)). CDNA cloning (Ferrier
et al., J. Mol. Endocrinol., 3:R1-R4 (1989)) and
immunocytochemical (Lukinius et al., Diabetologia,
20 32:240-244 (1989)) studies have demonstrated that amylin
is synthesized in the islet cells and stored in the islet
secretory granules along with insulin. It is cosecreted
with insulin (Kanatsuka et al., FEBS Lett., 259:199-201
(1989)). Low quantities of amylin have also been
25 detected in the stomach, intestine, lung and dorsal root
ganglion (Asai et al., Biochem. Biophys. Res. Commun.,
169:788-795 (1990)); and Ferrier et al., supra).

Biological investigations that followed the
isolation of amylin have shown that amylin inhibits basal
30 and insulin-stimulated glucose uptake as well as glycogen
synthesis by soleus muscles (Leighton et al., Nature,
335:632-635 (1988)). This peripheral insulin resistance
by amylin has also been demonstrated in vivo by
euglycemic glucose clamp studies with dogs (Sowa et al.,

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Diabetologia, 33:118-120 (1990)) and rats (Molina t al.,
Diabetes, 39:260-265 (1990)). Furthermore, thes
investigations in rats showed that amylin attenuated the
inhibition of hepatic glucose output by insulin (Molina
5 et al., supra). Based on these observations and the
finding that amylin inhibits basal insulin secretion
(Ohsawa et al., Biochem. Biophys. Res. Commun., 160:961-
967 (1989)), it has been suggested that amylin might play
a role in glucose metabolism and the pathophysiology of
10 noninsulin-dependent diabetes mellitus (NIDDM), commonly
known as Type II diabetes mellitus.

        Diabetes mellitus is a metabolic disease
characterized by chronic hyperglycemica, i.e., elevated
blood sugar levels. This disease affects a significant
15 percentage of the population. There are two major
categories of diabetes mellitus, commonly referred to as
Type I and Type II. In patients with Type I diabetes
mellitus, there is a loss of active β-cells in the islets
of Langerhans in the pancreas, resulting in low levels of
20 both insulin and amylin. Cooper, Medical Hypothesis,
26:284-288 (1991). Patients with Type I diabetes
mellitus who are treated with insulin frequently have a
tendency to develop hypoglycemia as a side effect. In
patients with Type II diabetes mellitus, there are
25 elevated levels of amylin. Patients with type II
diabetes mellitus display varying resistance to the
normal biological effects of insulin. Increased levels
of amylin, known as hyperamylinemia, have been implicated
in causing insulin resistance in a number of model
30 systems, including genetically obese LA/N-cp rats (Huang
et al., Hypertension, 19:i-101 - i-109 (1992)),
genetically obese diabetic yellow mice (Gill et al., Life
Sci, 48:703-718 (1991)), dexamethasone induced diabetic
rats (Jamal et al., J. Endocrin., 126:425-429 (1990)),
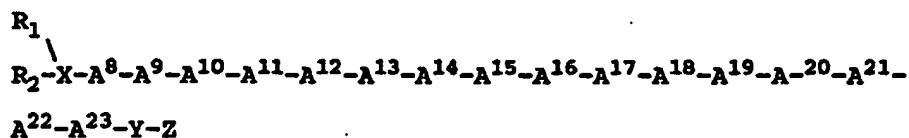35 streptozocin induced diabetic rats (Inoue et al.,

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Diabetes, _41_:723-727 (1992)), and ventr m dial
hyp thalamic lesioned rats and Zucker rats (Tokuyama et
al., _Endocrinology_, _128_:2739-2744 (1991)).

Other studies have shown that amylin, like

5   calcitonin, can exhibit serum calcium-lowering effects in
rats in vivo as well as in cell culture systems (Datta et
al., _Biochem. Biophys. Res. Commun._, _162_:876-881 (1989)).
Amylin has also been shown to act as an anorectic agent.
Balasubramaniam et al., _Peptides_, _12_:919-924 (1991).

10   Summary of the Invention

In general, the invention features amylin analogs
which behave as amylin antagonists and agonists.

In one aspect, the invention features amylin
analogs which are linear analogs of biologically active

15   amylin having the following amino acid formula:

$$R_2-X-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{-20}-A^{21}-$$

with R_1 branching above R_2;

$$A^{22}-A^{23}-Y-Z$$

20   wherein:

X is a chain of 0-5 amino acids, inclusive,
the N-terminal one of which is bonded to R_1
and R_2;

Y is a chain of 0-4 amino acids, inclusive,
25   the C-terminal one of which is bonded to Z;

Each of R_1, and R_2, independently, is H, C_1-
C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g.,
phenyl, naphthaleneacetyl), C_1-C_{12} acyl
(e.g., formyl, acetyl, and myristoyl), C_7-C_{18}
30   aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl
(e.g., _p_-methylphenyl);

A^8 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr,
Aib, or Anb;

A^9 is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or
35   N-Me-Thr;

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A^{10} is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

A^{11} is Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, or Lys;

A^{12} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{13} is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

A^{14} is Asn, Ala, Gln, Gly, N-Me-Asn, Nva, Aib, or Anb;

A^{15} is Phe, or any aromatic amino acid with or without substituents;

A^{16} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{17} is Val, Ile, Aib, Anb, or N-Me-Val;

A^{18} is His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

A^{19} is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A^{20} is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A^{21} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{22} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{23} is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR_3 or OR_3; wherein R_3 is H, C_1-C_{12} alkyl, C_7-C_{10} phenylalkyl, C_3-C_{20} alkenyl, C_3-C_{20} alkinyl, phenyl, or naphthyl.
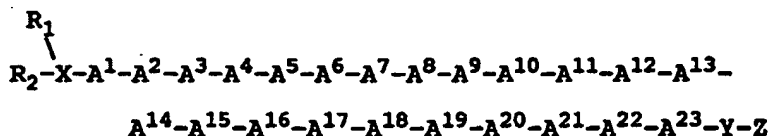
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In preferred embodiments, the analogs are antagonists.
In a highly preferred embodiment, the amylin antag nist
corresponds to the N-α acetyl derivative of amino acids 8
through 23 of human amylin with an amidated carboxy at
5   the C-terminus, referred to herein as N-α-ac-human amylin
(8-23)-NH_2, having the following formula:

N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-
Ser-Asn-Asn-Phe-NH_2 (SEQ ID NO:1)

In another preferred embodiment, the amylin antagonist
10 has the following formula:

N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-
Ser-Asn-Asn-Leu-NH_2. (SEQ ID NO:2)

In another aspect, the invention features amylin
analogs which are linear analogs of biologically active
15 amylin having the following amino acid formula:

$$R_2-X-A^1-A^2-A^3-A^4-A^5-A^6-A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-$$

$$A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-Y-Z$$

with R_1 bonded to X

20   wherein

X is a chain of 0-5 amino acids, inclusive,
the N-terminal one of which is bonded to R_1
and R_2;

Y is a chain of 0-4 amino acids, inclusive,
25   the C-terminal one of which is bonded to Z;

Each of R_1, and R_2, independently, is H,
C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} alyl,
C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^1 is Lys, Arg, homo-Arg, diethyl-homo-Arg,
30   Lys-ε-NH-R (where R is H, a branched or
straight chain C_1-C_{10} alkyl group, or an aryl
group), or Orn;

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A^2 is Cys, or Anb;

A^3 is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^4 is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A^5 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A^6 is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A^7 is Cys, or Anb;

A^8 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A^9 is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A^{10} is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

A^{11} is Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), or Orn;

A^{12} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{13} is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A^{14} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{15} is Phe, or any aromatic amino acid with or without substituents;

A^{16} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{17} is Val, Ile, Aib, Anb, or N-Me-Val;

A^{18} is His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, Ala, Aib, or Anb;

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A^{19} is Ser, Thr, N-Me-S r, N-Me-Thr, Ala, Aib, or Anb;

A^{20} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

5   A^{21} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{22} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{23} is Phe, any aromatic amino acid with or
10   without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR_3 or OR_3; wherein R_3 is H, C_1-C_{12} alkyl, C_7-C_{10} phenylalkyl, C_3-C_{20} alkenyl, C_3-C_{20} alkinyl, phenyl, or naphthyl.

15 In one highly preferred embodiment, the amylin analog
corresponds to amino acids 1 through 23 of human amylin
with an amidated carboxy at the C-terminus, referred to
herein as human amylin (1-23)-NH_2, having the following
formula:

20 Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-
Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH_2. (SEQ ID NO:3)

In another highly preferred embodiment, the amylin analog
corresponds to amino acids 1 through 23 of rat amylin,
with an amidated carboxy at the C-terminus, referred to
25 herein as rat amylin (1-23)-NH_2, having the following
formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-
Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH_2. (SEQ ID NO:4)

In yet another highly preferred embodiment, the amylin
30 analog corresponds to the derivative of amino acids 1
through 23 of rat amylin with α-amino normal butyric acid
substitutions at positions 2 and 7, and an amidated

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carboxy at the C-terminus, referred to herein as [Anb^{2,7}] rat amylin (1-23)-NH$_2$, having the following formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-
Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH$_2$. (SEQ ID NO:5)

5   In another aspect, the invention features a method
of treating Type II diabetes mellitus in a human being by
administering a therapeutic amount of an amylin
antagonist of the invention. In a highly preferred
method of treatment of Type II diabetes mellitus, N-α-ac-
10 human amylin (8-23)-NH$_2$ is adminstered.
           In another aspect, the invention features a method
of treating Type I diabetes mellitus in a human being by
administering a therapeutic amount of an amylin agonist
of the invention in conjunction with a therapeutic amount
15 of insulin.
           In still another aspect, the invention features a
method of treating hypercalcemia by administering a
therapeutic amount of an amylin agonist of the invention.
           The compounds of the invention exhibit a broad
20 range of biological activities, including those related
to glucose metabolism, calcium levels in the blood, and
appetite. Amylin antagonists of the invention attenuate
the inhibition by amylin of insulin-stimulated glucose
uptake. As a result, the amylin antagonists of the
25 invention act to reduce hyperglycemia resulting from
elevated levels of amylin associated with Type II
diabetes mellitus. Amylin agonists of the invention
inhibit insulin stimulated glucose uptake, thereby
tending to increase blood sugar levels. As a result, the
30 amylin agonists of the invention are useful in reducing
the hypoglycemia which frequently accompanies insulin
treatment of Type I diabetes mellitus. Amylin agonists
of the invention inhibit insulin stimulated glucose

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uptak , thereby tending t increase bl od sugar levels.
As a result, the amylin ag nists of the inventi n ar
useful in reducing the hypoglycemia which frequently
accompanies insulin treatment of Type I diabetes
5 mellitus. Amylin agonists of the invention also decrease
serum calcium levels, and are therefore useful for
treating hypercalcemia. In addition, amylin agonists
exhibit an appetite suppressant effect, while amylin
antagonists increase appetite. Amylin agonists and
10 antagonists are therefore useful in controlling food
intake. For example, amylin agonists are useful for
treating problems of overweight.

Many of the compounds of the invention are
especially advantageous because they are truncated
15 versions of the natural amylin peptide. The shorter
peptide not only facilitates easier synthesis and
purification of the compounds, but also improves
selectivity and reduces manufacturing procedures and
expenses.

20 Other features and advantages of the invention
will be apparent from the following description of the
preferred embodiments thereof, and from the claims.

Detailed Description
The drawings will first be briefly described.
25 ### Drawings
Fig. 1 shows the comparison of the primary
structures of human amylin (hAMYLIN) and rat amylin
(rAMYLIN).

Fig. 2 shows the effect of human amylin, and N-α-
30 ac-human amylin (8-23)-NH_2, separately and together, on
glucose uptake in C_2C_{12} muscle cells.

Fig. 3a and Fig. 3b show the in vivo effects of
saline, rat amylin, N-α-ac-human amylin (8-23)-NH_2, and
N-α-ac-human amylin (8-23)-NH_2 plus rat amylin on plasma

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gluc se levels, and plasma insulin levels, resp ctively,
in Sprague Dawley rats.

Fig. 4 shows the in vitro effect of human amylin
and human amylin (1-23)-NH_2, separately, on insulin
5 stimulated glucose uptake in C_2C_{12} muscle cells.

Fig. 5a and 5b show the in vivo effects of saline,
rat amylin, human amylin (1-23)-NH_2, and human amylin (1-
23)-NH_2 plus rat amylin on plasma glucose levels, and
plasma insulin levels, respectively, in Sprague Dawley
10 rats.

Fig. 6 shows the in vitro effects of rat amylin
(1-23)-NH_2 and [Anb^{2,7}] rat amylin (1-23)-NH_2, separately,
on insulin stimulated glucose uptake in C_2C_{12} muscle
cells.

15   Fig. 7a and 7b show the in vivo effects of saline,
rat amylin, [Anb^{2,7}] rat amylin (1-23)-NH_2, and [Anb^{2,7}]
rat amylin (1-23)-NH_2 plus rat amylin on plasma glucose
levels, and plasma insulin levels, respectively, in
Sprague Dawley rats.

20   Structure

The sequences of naturally occuring human amylin
("hAmylin") and rat amylin ("rAmylin") are set forth in
Fig. 1. Balasubramaniam et al., Peptides, 12:919-924
(1991). There is a high degree of sequence homology
25 between amylin from these two species. It is believed
that in naturally occuring hAmylin and rAmylin, the
cysteine residues at positions 2 and 7, present in both
species, form an internal disulfide bond, resulting in a
cyclic structure.

30   The amylin analogs of the invention are based upon
the biologically active subfragments comprising amino
acids 8-23 of hAmylin and rAmylin and derivatives
thereof; and upon the biologically active subfragments
comprising amino acids 1-23 of hAmylin and rAmylin and

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derivativ s thereof. In the amylin anal g formulas s t
f rth herein, the symbols A^x and the like; and Ser, Leu
and the like, as found in a peptide sequence herein,
stand for amino acid residues. When an amino acid
5 residue is optically active, it is the L-form
configuration that is intended unless the D-form is
expressly designated. All peptide sequences mentioned
herein are written according to the usual convention
whereby the N-terminal amino acid is on the left and the
10 C-terminal amino acid is on the right. A short line
between two amino acid residues indicates a peptide bond.
An -OR or an -NHR substituent on the carboxy terminal end
of a peptide replaces the -OH on the carboxy terminal
amino acid residue, yielding -NH-CH(R)-COOR, and -NH-
15 CH(R)-CONHR as the C-terminal residues, respectively.
When the carboxy terminal substituent is -NH$_2$, the
peptide is in the amidated carboxy form.

    As set forth above and for convenience in
describing this invention, the conventional and
20 nonconventional abbreviations for the various amino acids
are used. They are familiar to those skilled in the art;
but for clarity are listed below.

    Asp = D = Aspartic Acid
    Ala = A = Alanine
25 Arg = R = Arginine
    Asn = N = Asparagine
    Cys = C = Cysteine
    Gly = G = Glycine
    Glu = E = Glutamic Acid
30 Gln = Q = Glutamine
    His = H = Histidine
    Ile = I = Isoleucine
    Leu = L = Leucine
    Lys = K = Lysine

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  Met =  M  =  M thi nine
  Phe =  F  =  Phenylalanine
  Pro =  P  =  Proline
  Ser =  S  =  Serine
5 Thr =  T  =  Threonine
  Trp =  W  =  Tryptophan
  Tyr =  Y  =  Tyrosine
  Val =  V  =  Valine
  Orn =  Ornithine
10 Nal =  2-napthylalanine
  Nva =  norvaline
  Thi =  2-thienylalanine
  Pcp =  4-chlorophenylalanine
  Bth =  3-benzothienyalanine
15 Bip =  4,4'-biphenylalanine
  Tic =  tetrahydroisoquinoline-3-carboxylic acid
  Aib =  aminoisobutyric acid
  Anb =  α-aminonormalbutyric acid
  Dip =  2,2-diphenylalanine
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20   The compounds of the present invention can be
provided in the form of pharmaceutically acceptable
salts.   Examples of preferred salts are those with
therapeutically acceptable organic acids, e.g., acetic,
lactic, maleic, citric, malic, ascorbic, succinic,
25 benzoic, salicylic, methane sulfonic, toluene sulfonic,
trifluoroacetic, or pamoic acid, as well as polymeric
acids such as tannic acid or carboxymethyl cellulose, and
salts with inorganic acids, such as the hydrohalic acids,
e.g., hydrochloric acid, sulfuric acid, or phosphoric
30 acid and the like.

Analysis

       The structure-activity relationships of amylin and
amylin analogs were studied both in an in vitro model
using a mouse muscle cell line, C_2C_{12}, and an in vivo
35 model using Sprague Dawley rats.

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In the in vitro studies, insulin stimulated the glucose uptake by th C_2C_{12} cell line in a dose-dependent manner and this was attenuated by rat amylin (100 pM). However, rat amylin did not exhibit any effect on the
5 basal glucose uptake by this cell line. Cholera toxin did not have any effect on insulin stimulated glucose uptake but blocked the inhibitory effect of rat amylin.

Several partial sequences of human and rat amylin and their analogs were synthesized and their effects
10 investigated in the in vitro and in vivo models.

Peptide Synthesis

Human and rat amylin were synthesized according to the procedures set forth in Balasubramaniam et al., Peptides, 12:919-924 (1991). The synthetic peptides were
15 characterized by sequence and mass spectral analyses, and were found to be greater than 97% pure by analytical reversed-phase chromatography.

Peptide synthesis was accomplished on an Applied Biosystem Model 430A synthesizer. HPLC was carried out
20 on a Waters Model 600 solvent delivery system in conjunction with a U6K injector, Model 481 spectrophotometer and Baseline 810 Data collection software in an IBM-XT computer. Protected amino acid derivatives (Peninsula, CA), synthesis reagents (Applied
25 Biosystems, CA) and solvents (Fischer Scientific, OH) were obtained commercially and used without further purification. Paramethylbenzhydroxylamine (MBHA) resin (0.45 mmol, NH_2 group) was placed in the reaction vessel of the synthesizer and the amino acid derivatives were
30 coupled automatically using the standard program provided by the manufacturers modified to incorporate a double

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coupling procedure. All amino acids were coupled using
2.2 equivalents of pref rm d symmetrical anhydrides.
Arg, Asn, and Gln, however, were coupled as preformed 1-
hydroxybenzotriazole esters (4.4 equivalent) to avoid

5 deamidations or lactam formation. At the end of the
synthesis the N-α-Boc group was removed, and the peptide
resin (1.3 g) was treated with hydrogen fluoride (~10/ml)
containing dimethylsulfide (~0.8 ml), p-cresol (~0.8 g)
and p-thiocresol (~0.2 g) for one hour at -2 to 4°C. HF

10 was evacuated and the residue transferred to a fritted
filter funnel with diethyl ether, washed repeatedly with
diethyl ether, extracted with acetic acid (2X15 ml) and
lyophilized. The crude peptide (100 mg) thus obtained
was dissolved in 6 M guanidine HCl (6 ml), diluted with

15 500 ml of distilled water and the Ph adjusted to 8 with
ammonia. A solution of 0.1% potassium ferricyanide (w/v)
was then added gradually with constant stirring until a
permanent yellow color persisted. After stirring for an
additional 30 minutes, the Ph of the solution was

20 adjusted to 5 with acetic acid. The solution was then
stirred with anion-exchange resin (AG-3, Cl⁻ form, 10 g
wet weight) for 30 minutes, filtered through 0.45 microns
filter and pumped into a semipreparative reversed phase
column and purified as described in Balasubramaniam et

25 al., _Peptides_, _12_:919-924 (1991). The overall yield of
rat and human amylin thus obtained varied between 10-20%.

In Vitro Assays

C_2C_{12} cells were cultured at 37°C in a humidified
5% CO_2 atmosphere, in low glucose (1 g/l) DMEM medium

30 containing 20% fetal bovine serum, and 0.5% chick embryo
extract (growth medium). Cells were seeded in 75-cm^2
flasks at a density of 1x10^6 cells per flask. When the
cells became confluent (3-4 days), they were trypsinized
(0.25% trypsin) and washed with growth medium. The final

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cell pellet was suspended in growth medium and seeded at a density of 2.5-10⁴ cells/well into 24 well plates (16 mm diameter) and allowed to grow to 70% confluence (3 days). To induce fusion, the mononucleated myoblasts were exposed to medium containing 10% horse serum instead of 20% FBS (fusion medium). Fusion media was changed every day to avoid the premature detachment of cells and the cells were almost completely fused into multinucleated myotubes by the 9th day (6 days in fusion medium). Medium was changed one day before the experiment.

2-deoxyglucose uptake in C₂C₁₂ myotubes was determined as described in Klip et al., Biochem. J., 242:131-136 (1987). In brief, cells were washed with PBS (phosphate-buffered saline) and incubated for 5h in the serum-free, high-glucose (25 mM) DMEM medium. At the end of incubation, cells were washed with PBS and different doses of amylin or amylin analogs (10pM- 10µM) were added and incubated with 2-deoxy-[³H]-glucose (1mM) for 10 min. Non-carrier-mediated uptake was determined by incubating the cells with cytochalasin B (15 µM). Uptake was terminated by rapidly aspirating the solution, and cells were washed with ice-cold PBS. Cell-associated radioactivity was determined by lysing the cells in 1 M NaOH and the aliquots were neutralized and counted in a scintillation counter. Protein content of the aliquots was determined by the Lowry method.

After seeding, the undifferentiated mononucleated myoblasts grew logarithmically and reached 70% confluence by day 3. Fused cells were detected by day 5 and contained >90% multinucleated myotubes by day 9 (6 days in fusion media). In 6-day-old cells there was a 30% increase in glucose uptake in response to insulin compared to a 68-115% increase in 9-day-old cells. These results are similar to earlier observations (Klip et al.,

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<u>supra</u>). The low insulin response by 6-day-old cells,
presumably, is due to the presence of undifferentiated
myoblasts with low insulin-receptor density as evident in
the L_6 muscle cell line (Beguinot et al., <u>Endocrinology</u>,
5 <u>18</u>:446-455 (1986)). Because of these findings, and the
observation that insulin stimulated glucose uptake in 9-
day-old cells in a dose-dependent manner, we used 9-day-
old C_2C_{12} cells to test the effects of amylin or amylin
analogs on insulin-stimulated glucose uptake. The
10 maximal insulin-stimulated response was observed at 100
nM and remained plateaued at further increasing doses.
The insulin-stimulated glucose uptake in C_2C_{12} myotubes
appears to occur mainly through facilitated diffusion
because cytochalasin B(15 μM) inhibited >90% of insulin-
15 stimulated 2-deoxyglucose uptake by the cells.

<div align="center">In Vivo Assays</div>

Sprague Dawley rats (Zivic Miller, Zelienople, PA)
used in this investigation were housed individually in
air-conditioned rooms (22-24°C) under 12-hour light/dark
20 cycle with ad lib access to Purina rat chow and water.

Sprague Dawley rats weighing about 300g were
fasted overnight (18-22 hrs). Rats were then
anesthetized with sodium pentobarbital (40 mg/kg) and
catheters were implanted in the jugular vein.   Saline
25 (0.1 ml), rat amylin (50 μg) in saline (0.1ml) or peptide
fragments/analogs (100μg) in saline (0.1 ml) were
injected through the jugular vein and then flushed with
another 0.1 ml of saline. In the cases of studying
antagonistic effects, injection of peptide
30 fragments/analogs (100μg) in saline (0.1 ml) were
followed 2 min. later with rat amylin (50 μg) in saline
(0.1 ml) injection. 30 min. after the injection of the
peptides, blood (4-5 ml) was drawn through the jugular
vein and collected in heparinized tubes containing
35 aprotinin (10 μl). Plasma was obtained by

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centrifugation. Plasma glucose and insulin levels were
det rmined by the gluc se oxidase method (Model 27
glucose analyzer, Yellow Springs Instruments, Yellow
Springs, OH) and a radioimmunoassay kit (Peninsula
5 Laboratories, Belmont, CA), respectively.

Results

Referring to Fig. 2, one of the antagonists of the
invention, N-α-ac-human amylin(8-23)-NH_2, exhibited no
significant effect on insulin stimulated glucose uptake
10 in the in vitro assay when tested separately. Still
referring to Fig. 2, the presence of N-α-ac-human amylin
(8-23)-NH_2 (1μM) with human amylin consistently shifted
the inhibitory dose-response curve of human amylin on
insulin stimulated glucose uptake to the right (i.e.,
15 higher concentrations of human amylin), increasing the
IC_{50} value from 0.20 nM to 350 nM.

In vivo effects of N-α-ac-human amylin (8-23)-NH_2
were investigated in anesthetized (45 mg/kg) Sprague
Dawley rats (~300 g) fasted overnight (≥ 20 h). The
20 following samples were injected via a cannulated jugular
vein into individual rats: (1) 100 μl of saline (n = 5),
(2) rat amylin (50μg), (3) N-α-ac-human amylin (8-23)-NH_2
(100 μg), and (4) N-α-ac-human amylin (8-23)-NH_2 (100μg)
followed 2 min later by rat amylin (50μg). Thirty
25 minutes after injection, 4-5 ml blood was collected in
heparinized tubes from each of the rats and the plasma
separated by centrifugation. Plasma glucose and insulin
levels were subsequently determined, and the results are
set forth in Fig. 3a and 3b, respectively.
30 Referring to Fig. 3a, rat amylin significantly
increased the plasma glucose level compared to the saline
control, while N-α-ac-human amylin (8-23)-NH_2
significantly decreased the plasma glucose levels
relative to the control, probably by antagonizing the

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effects of endogenous amylin. Still referring to Fig.
3a, N-α-ac-human amylin (8-23)-NH$_2$ significantly
attenuated the elevation of plasma glucose by rat amylin
in the rat which received both N-α-ac-human amylin (8-
5 23)-NH$_2$ and rat amylin (i.e. plasma glucose levels were
brought down near the control value). The p values in
Fig. 3a and 3b, and throughout, refer to values obtained
using the ANOVA program with n equal to 5 to 8.

These observations confirm that N-α-ac-human
10 amylin (8-23)-NH$_2$ is a potent antagonist of human amylin
in vitro, and of rat amylin in vivo.

Referring to Fig. 4, human amylin (1-23)-NH$_2$
inhibited insulin stimulated glucose uptake in the in
vitro assay in a manner similar to human amylin. Still
15 referring to Fig. 4, human amylin (1-23)-NH$_2$ exhibited a
dose-response inhibitory effect on insulin-stimulated
glucose uptake by C$_2$C$_{12}$ cells with a potency comparable to
that of intact human amylin.

Referring to Fig. 5a, human amylin (1-23)-NH$_2$
20 attenuated rat amylin induced hyperglycemia.

Referring to Fig. 6, [Anb^{2,7}] rat amylin(1-23)-NH$_2$
inhibited the insulin stimulated glucose uptake in the in
vitro assay in a manner qualitatively similar to rat
amylin(1-23)-NH$_2$. Referring to Fig. 6 and Fig. 4, rat
25 amylin (1-23)-NH$_2$ exhibited a dose-response inhibitory
effect on insulin-stimulated glucose uptake by C$_2$C$_{12}$ cells
with a potency comparable to that of intact human amylin.
Still referring to Fig. 6 and Fig. 4, [Anb^{2,7}] rat amylin
(1-23)-NH$_2$ also exhibited a potency comparable to that of
30 human amylin.

Referring to Fig. 7, [Anb^{2,7}] rat amylin(1-23)-NH$_2$
had no significant effect on amylin induced
hyperglycemia, but the tendency was in the direction of
attenuation.

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The results obtained t gether with reported data
in the literature ar set forth in Table 1 b low.

PEPTIDES	C_2C_{12}-effect on insulin stimulated glucose uptake[1]	ANESTHETIZED RATS PLASMA GLUCOSE[1,2]	plasma Ca^{2+}
1. human amylin ("HA")	inhibits (Fig. 2)	elevates[3]	lowered[4]
2. rat amylin ("RA")	inhibits[5]	elevates[3]	lowered[4]
3. HA(1-23)-NH₂	inhibits (similar to human amylin) (Fig. 4)	1. lowers basal (Fig. 5a) 2. attenuates amylin induced hyperglycemia	N.D.
4. RA(1-23)-NH₂	inhibits (similar to human amylin) (Fig. 6)	N.D.	N.D.
5. [Anb 2,7]RA(1-23)-NH₂	inhibits (similar to human amylin) (Fig. 6)	1. lowers basal (Fig. 7a) 2. no effect on amylin induced hyperglycemia	N.D.
6. N-α-Ac-HA((8-23)-NH₂	1. no effect 2. attenuates amylin effects (Fig. 2)	1. lowers basal (Fig. 3a) 2. attenuates amylin induced hyperglycemia.	N.D.

1. Present study; 2. effects of 100 μg peptide analogs on basal or 50 μg rat amylin induced hyperglycemia; 3. Molina et al., Diabetes, 39:260-265 (1990); and Young et al., Am. J. Physiology, 259: E457-461 (1990); 4. Data et al., Biochem. Biophys. Res. Commun., 162:876-881 (1989); 5. Sheriff et al., Biochim. Biophys. Acta, 1136: 219-222 (1992).

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The agonist or antagonist effect of other amylin
analogs f the inv ntion may be determined by the assays
described above.

USE

Amylin inhibits insulin stimulated glucose uptake
and glycogen synthesis, and increases the hepatic glucose
output. Therefore, it appears that a particular ratio of
insulin to amylin is required to maintain the normal
plasma glucose levels.

The amylin agonists and antagonists of the
invention have useful applications in treating Type I and
II diabetes mellitus, respectively. Since humans with
Type II diabetis mellitus have elevated levels of amylin
and elevated blood glucose levels, administration of an
amylin antagonist of the invention in an amount
sufficient to decrease blood glucose levels to normal or
clinically acceptable levels provides therapeutic
results. Humans with Type I diabetis mellitus have
decreased levels of both insulin and amylin, and when
treated with insulin have a tendency to develop
hypoglycemia. Administration of an amylin agonist of the
invention in an amount sufficient to increase blood
glucose levels to normal or clinically acceptable levels
in response to insulin induced hypoglycemia, together
with a therapeutic amount of insulin, provides
therapeutic results.

Amylin agonists of the invention decrease serum
calcuim levels and may be administered to humans to treat
hypercalcemia. Amylin agonists of the invention exhibit
an appetite suppressant effect, while amylin antagonists
increase appetite. Amylin agonists and antagonists of
the invention are therefore useful in controlling food
intake. For example, amylin agonists of the invention
may be administered for the treatment of obesity.

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The peptides f th inventi n may be administered
t a human in on of the traditional modes (e.g., orally,
parenterally, transdermally, or transmucosally), or in a
sustained release formulation using a biodegradable
biocompatible polymer.

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<u>SEQUENCE LISTING</u>

(1) GENERAL INFORMATION:

    (i) APPLICANT: A. Balasubramaniam

    (ii) TITLE OF INVENTION: AMYLIN ANTAGONISTS AND AGONISTS

    (iii) NUMBER OF SEQUENCES: 7

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: Fish & Richardson
        (B) STREET: 225 Franklin Street
        (C) CITY: Boston
        (D) STATE: Massachusetts
        (E) COUNTRY: U.S.A.
        (F) ZIP: 02110-2804

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
        (B) COMPUTER: IBM PS/2 Model 50Z or 55SX
        (C) OPERATING SYSTEM: MS-DOS (Version 5.0)
        (D) SOFTWARE: WordPerfect (Version 5.1)

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (vii) PRIOR APPLICATION DATA:

        (A) APPLICATION NUMBER: 08/060,265
        (B) FILING DATE: 12 May 1993

    (viii) ATTORNEY/AGENT INFORMATION:

        (A) NAME: Clark, Paul T.
        (B) REGISTRATION NUMBER: 30,162
        (C) REFERENCE/DOCKET NUMBER: 00537/078WO1

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        (A) TELEPHONE: (617) 542-5070
        (B) TELEFAX: (617) 542-8906
        (C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

    (i) SEQUENCE CHARACTERISTICS:.

        (A) LENGTH: 16
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

N α Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn Phe NH$_2$
         5   10   15

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 16
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

N α Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val Arg Ser Ser Asn Asn Leu NH$_2$
         5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 23
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
         5   10   15
Val His Ser Ser Asn Asn Phe NH$_2$
       20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 23
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
         5   10   15
Val Arg Ser Ser Asn Asn Leu NH$_2$
       20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 23
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Lys Anb Asn Thr Ala Thr Anb Ala Thr Gln Arg Leu Ala Asn Phe Leu
         5   10   15
Val Arg Ser Ser Asn Asn Leu NH$_2$
       20

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 37
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
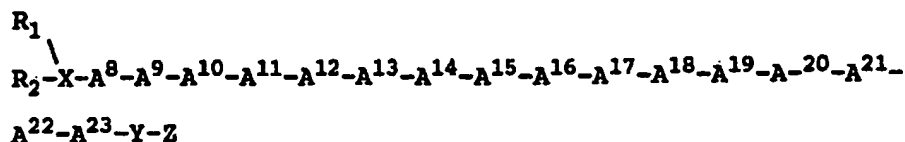   (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
                  5                   10                  15
Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
            20                  25                  30
Gly Ser Asn Thr Tyr
            35
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 37
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
                  5                   10                  15
Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val
            20                  25                  30
Gly Ser Asn Thr Tyr
            35
```

What is claimed is:

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1. An amylin analog f the amin acid formula:

R_1
\
R_2-X-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A-^{20}-A^{21}-

A^{22}-A^{23}-Y-Z

wherein:

X is a chain of 0-5 amino acids, inclusive,
the N-terminal one of which is bonded to R_1
and R_2;

Y is a chain of 0-4 amino acids, inclusive,
the C-terminal one of which is bonded to Z;

Each of R_1, and R_2, independently, is H, C_1-
C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g.,
phenyl, naphthaleneacetyl), C_1-C_{12} acyl
(e.g., formyl, acetyl, and myristoyl), C_7-C_{18}
aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl
(e.g., p-methylphenyl);

A^8 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr,
Aib, or Anb;

A^9 is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or
N-Me-Thr;

A^{10} is Gln, Ala, Asn, N-Me-Gln, Gly, Nva,
Aib, or Anb;

A^{11} is Arg, homo-Arg, diethyl-homo-Arg, Lys-
ε-NH-R (where R is H, a branched or straight
chain C_1-C_{10} alkyl group, or an aryl group),
Orn, or Lys;

A^{12} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{13} is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-
Thr, Aib, or Anb;

A^{14} is Asn, Ala, Gln, Gly, N-Me-Asn, Nva,
Aib, or Anb;

A^{15} is Phe, or any aromatic amino acid with
or without substituents;

A^{16} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

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A^{17} is Val, Ile, Aib, Anb, r N-M -Val;

A^{18} is His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

A^{19} is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A^{20} is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A^{21} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{22} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{23} is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR_3 or OR_3; wherein R_3 is H, C_1-C_{12} alkyl, C_7-C_{10} phenylalkyl, C_3-C_{20} alkenyl, C_3-C_{20} alkinyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

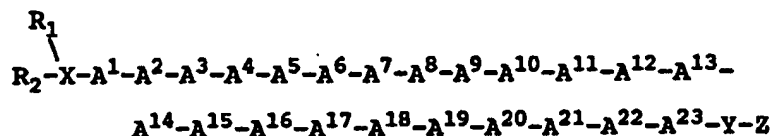2.   An amylin analog of claim 1 which is an antagonist.

3.   An amylin analog of claim 2 corresponding to the N-α-acetyl derivative of amino acids 8 through 23 of human amylin with an amidated carboxy at the C-terminus ("N-α-ac-human amylin (8-23)-NH_2") having the formula: N-α-ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH_2, or a pharmaceutically acceptable salt thereof.

4.   An amylin analog of claim 2 having the amino acid formula:

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N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-
Ser-Asn-Asn-Leu-NH$_2$, or a pharmac utically acceptable
salt thereof.

    5. An amylin analog of the amino acid formula:

$$R_2-X-A^1-A^2-A^3-A^4-A^5-A^6-A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-$$

with R_1 bonded above R_2,

$$A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-Y-Z$$

wherein

        X is a chain of 0-5 amino acids, inclusive,
the N-terminal one of which is bonded to R_1
and R_2;

        Y is a chain of 0-4 amino acids, inclusive,
the C-terminal one of which is bonded to Z;

        Each of R_1, and R_2, independently, is H,
C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} alyl,
C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

        A^1 is Lys, Arg, homo-Arg, diethyl-homo-Arg,
Lys-ε-NH-R (where R is H, a branched or
straight chain C_1-C_{10} alkyl group, or an aryl
group), or Orn;

        A^2 is Cys, or Anb;

        A^3 is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb,
or Nva;

        A^4 is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib,
or Anb;

        A^5 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala,
Aib, or Anb;

        A^6 is Thr, Ser, N-Me-Ser, or N-Me-Thr, Ala,
Aib, or Anb;

        A^7 is Cys, or Anb;

        A^8 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala,
Aib, or Anb;

        A^9 is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib,
or Anb;

A^{10} is Gln, Ala, Asn, N-M -Gln, Gly, Nva, Aib, or Anb;

A^{11} is Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), or Orn;

A^{12} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{13} is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A^{14} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{15} is Phe, or any aromatic amino acid with or without substituents;

A^{16} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{17} is Val, Ile, Aib, Anb, or N-Me-Val;

A^{18} is His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, Ala, Aib, or Anb;

A^{19} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A^{20} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A^{21} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{22} is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A^{23} is Phe, any aromatic amino acid with or without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR_3 or OR_3; wherein R_3 is H, C_1-C_{12} alkyl, C_7-C_{10} phenylalkyl, C_3-C_{20} alkenyl, C_3-C_{20} alkinyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

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6.   An amylin analog of claim 5 corresponding to amino acids 1 through 23 of human amylin with an amidated carboxy at the C-terminus ("human amylin (1-23)-NH$_2$") having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH$_2$, or a pharmaceutically acceptable salt thereof.

7.   An amylin analog of claim 5 corresponding to amino acids 1 through 23 of rat amylin, with an amidated carboxy at the C-terminus ("rat amylin (1-23)-NH$_2$") having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH$_2$, or a pharmaceutically acceptable salt thereof.

8.   An amylin analog of claim 5 corresponding to the derivative of amino acids 1 through 23 of rat amylin with α-amino normal butyric acid substitutions at positions 2 and 7, and an amidated carboxy at the C-terminus ("[Anb^{2,7}] rat amylin (1-23)-NH$_2$") having the formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH$_2$, or a pharmaceutically acceptable salt thereof.

9.   A method of treating Type II diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin antagonist of claim 2.

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10. The method of claim 9 in which said amylin antagonist is N-α-ac-human amylin (8-23)-NH$_2$.

11. A method of treating Type I diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist, and a therapeutic amount of insulin.

12. A method of treating hypercalcemia in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist.

13. A method of controlling food intake in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 1 or claim 5.

```
1                      5                    10                    15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe

16                     20                    25                    30
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr

31                     35
Asn Val Gly Ser Asn Thr Tyr

                    hAMYLIN (SEQ ID NO:6)


1                      5                    10                    15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe

16                     20                    25                    30
Leu Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr

31                     35
Asn Val Gly Ser Asn Thr Tyr

                    rAMYLIN (SEQ ID NO:7)
```
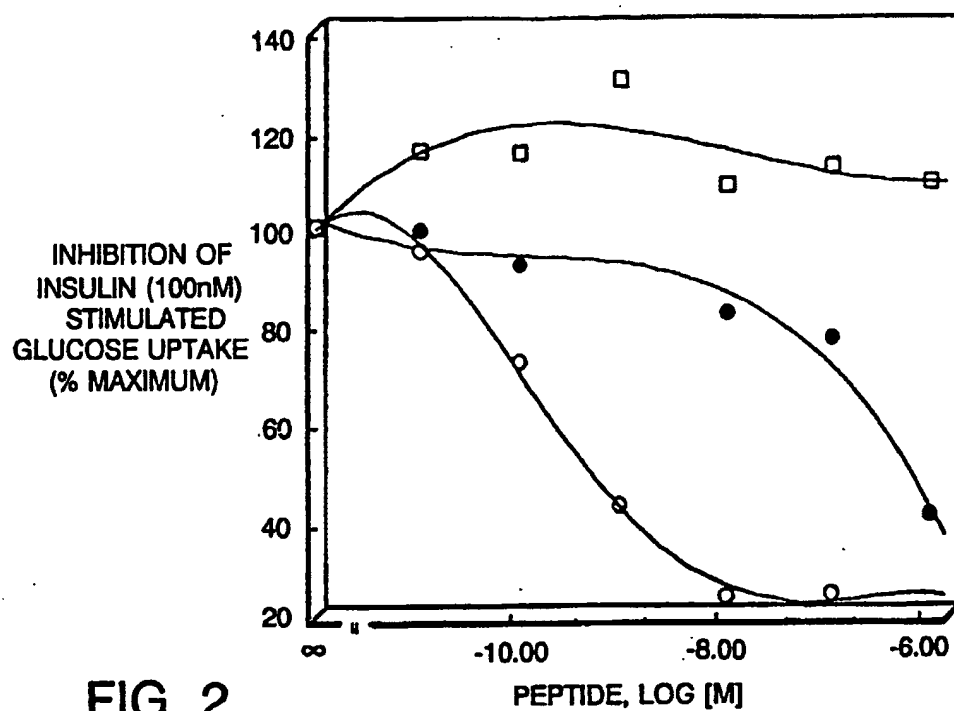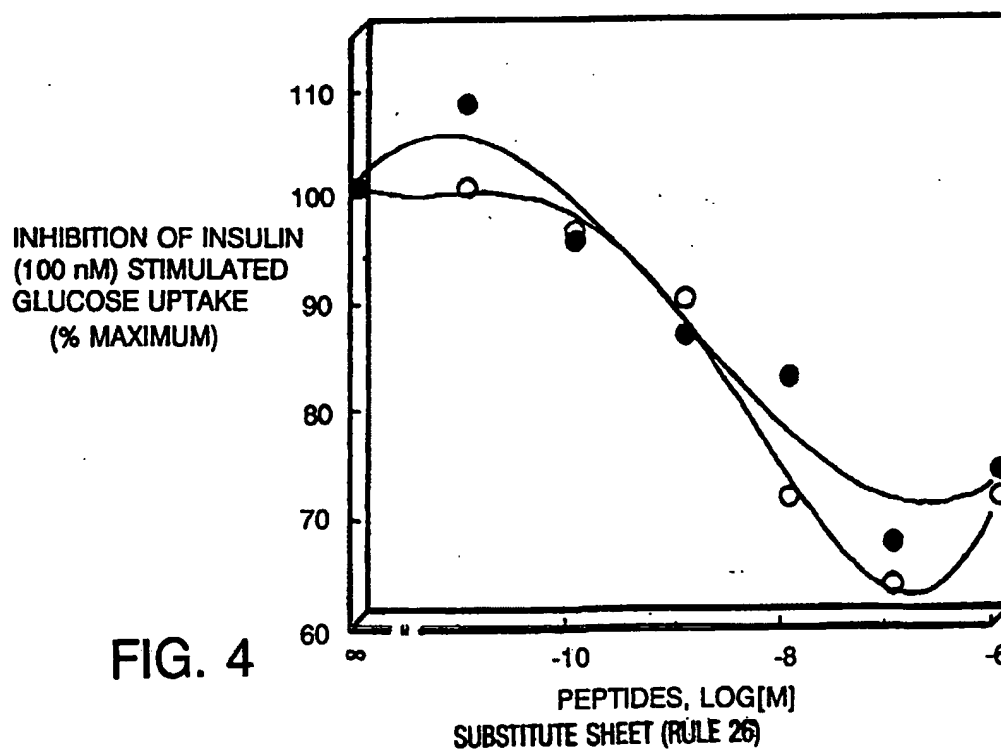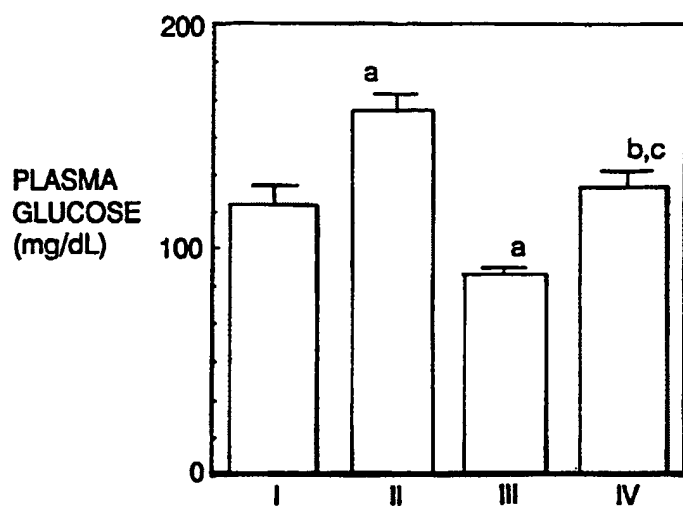
FIG. 1

INHIBITION OF
INSULIN (100nM)
STIMULATED
GLUCOSE UPTAKE
(% MAXIMUM)

PEPTIDE, LOG [M]

FIG. 2

INHIBITION OF INSULIN
(100 nM) STIMULATED
GLUCOSE UPTAKE
(% MAXIMUM)

PEPTIDES, LOG[M]

FIG. 4

FIG. 3A

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin

I= Saline
II= Rat amylin (50μg)
III= N-α-ac-human
amylin (8-23)-nh2 (100μg)
IV= N-α-ac-human amylin
(8-23)-NH^2 (100μg) plus
rat amylin (50μg)
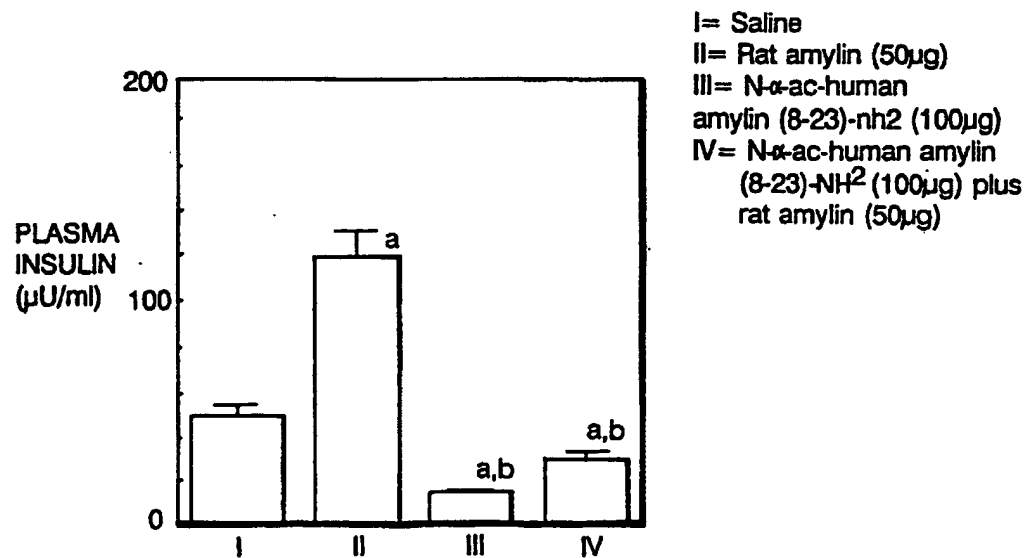
FIG. 3B

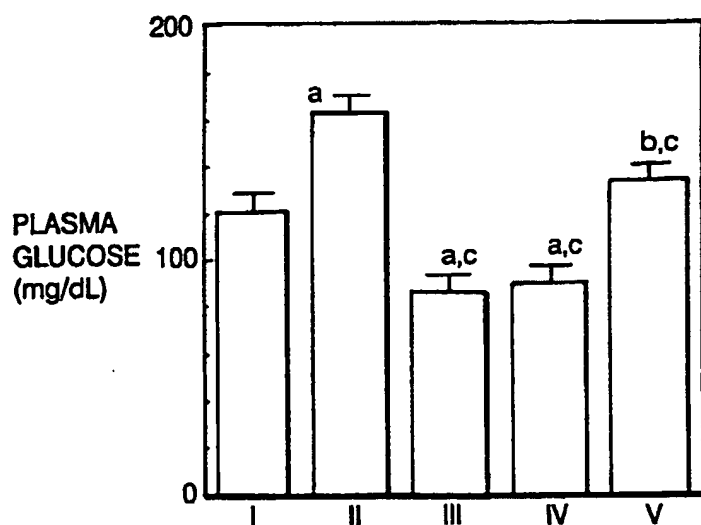a= p< 0.05 vs control
b= p< 0.05 vs rat amylin

FIG. 5A

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin

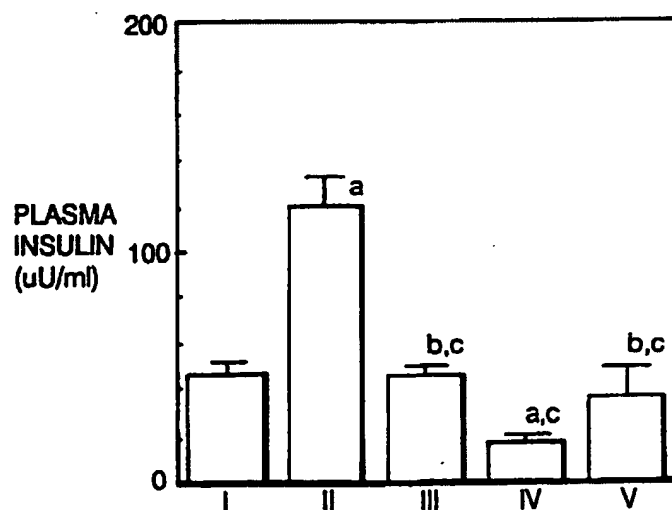FIG. 5B

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin

I= Saline
II= Rat amylin (50µg)
III= Human amylin (1-23)-NH$_2$ (50µg)
IV= Human amylin (1-23)-NH$_2$ (100µg)
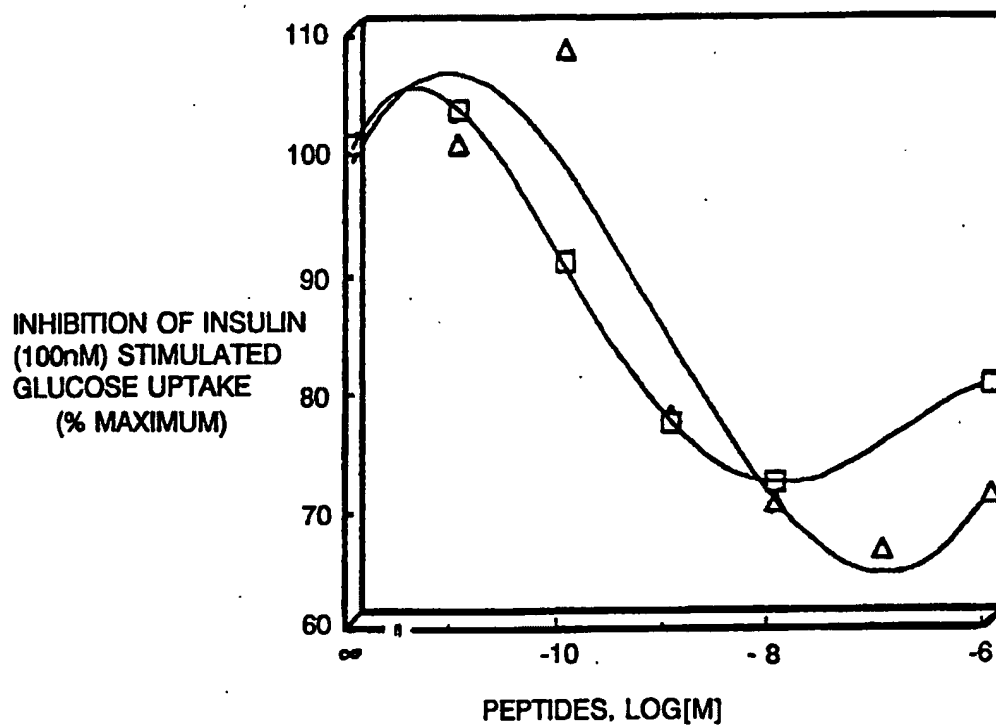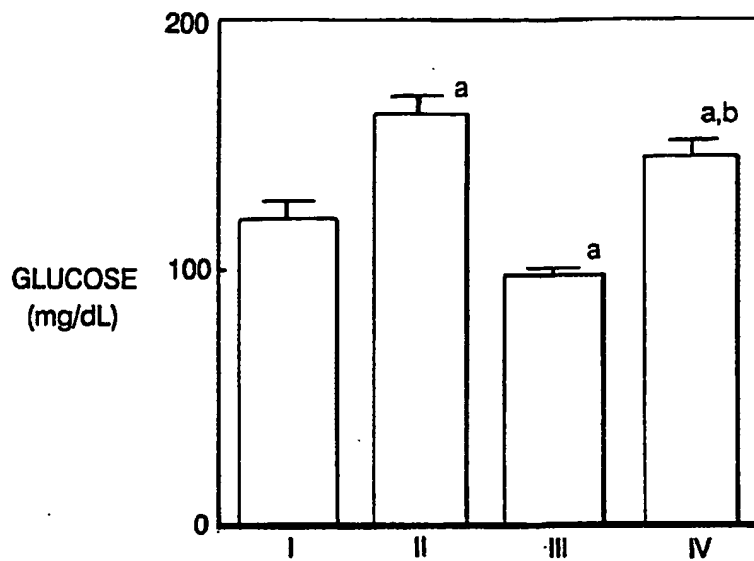V= Human amylin (1-23)-NH$_2$ (100µg)
plus rat amylin (50µg)

FIG. 6

FIG. 7A
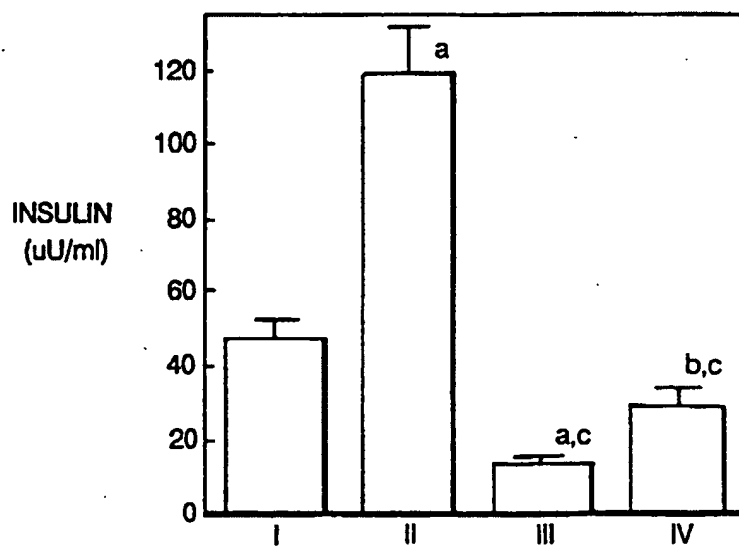
a= p<0.5 vs control
b= not significant vs rat amylin

FIG. 7B

a= p< 0.05 vs control
b= not significant vs control
c=p< 0.05 vs rat amylin

I= Saline
II= Rat amylin (50μg)
III= [Anb ^{2,7}] rat amylin
(1-23)-NH^2 (100μg)
IV= [Anb^{2,7}] rat amylin (1-23)-NH^2
(100μg) plus Rat amylin (50μg)

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 37/02; C07K 7/06, 7/08
US CL :530/326; 514/13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/326; 514/13
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Science USA, Vol. 85, issued 1988, Cooper et al , "Amylin found in amyloid deposits in Human Type 2 Diabetes Mellitus may be Hormone that Regulates Glycogen Metabolism in Skeletal Muscle", pages 7763-7766, see entire document.	1-13
A	Proceedings of the National Academy of Science USA, Vol. 84, issued June 1987, Westermark et al, "Amyloid Fibrils in Human Insulinoma and Islets of Langerhans of the Diabetic Cat Are Derived From A Neuropeptid-like Protein Also Present in Normal Islet Cells", pages 3881-3885, see entire document.	1-13

[X] Further documents are listed in the continuation of Box C.	[ ] See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
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"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed

"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 AUGUST 1994	15 AUG 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	S.G. Marshall Jill Warden for
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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Biochemical and Biophysical Research Communications, Vol. 160, No. 2, issued 28 April 1989, Ohsawa et al, "Islet Amyloid Polypeptide Inhibits Glucose-Stimulated Insulin Secretion From Isolated Rat Pancreatic Islets", pages 961-967. see entire document.	1-13
A,P	US, A, 5,266,561 (COOPER ET AL) 30 November 1993, see entire document.	1-13